A Computational Model of In Vitro Breast Cancer Cells Spheroid Formation

F. Lignet [1,3], A. Emde [2], Y. Yarden [2], R.M.H. Merks [3,4,5], B. Ribba [1]
[1] INRIA Rhône-Alpes, and Unité de Mathématiques Pures et Appliquées, CNRS, UMR5669 ENS de Lyon, France; [2] Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100 Israel; [3] Centrum Wiskunde & Informatica, 1098 XG Amsterdam, The Netherlands; [4] Netherlands Institute for Systems Biology / Netherlands Consortium for Systems Biology; [5] Mathematical Institute, Leiden University, Leiden, The Netherlands

Context

The HER2 receptor is over-expressed in 30 % of breast cancers and is associated to poor survival prognosis. A better understanding of carcinogenesis mechanisms would permit to improve the efficacy of existing treatments. In vitro experiments offer a good insight on the formation of normal and mutated tumour spheroids. Computational analysis can be used to test hypothesis on the cellular processes involved.
In this work, we built a "cell-based" model to study the importance of cell proliferation, polarization and apoptosis in breast tumour spheroid formation.

A cell-based model

Our modelling is performed with the cell-based simulation environment CompuCell3D which implements the Cellular Potts Model (CPM) [2, 3].
The CPM represents cells as connected domains of lattice points. At each time step, to represent amoeboid cell motility, the algorithm attempts to randomly extend or retract the domains. The acceptance probability of these attempts depends on the adhesive interactions with surrounding cells and the extracellular matrix, and on a parameter that represents the intrinsic cell motility. Cells properties such as adhesion, proliferation rate, or growth rate can be defined by the modeller.

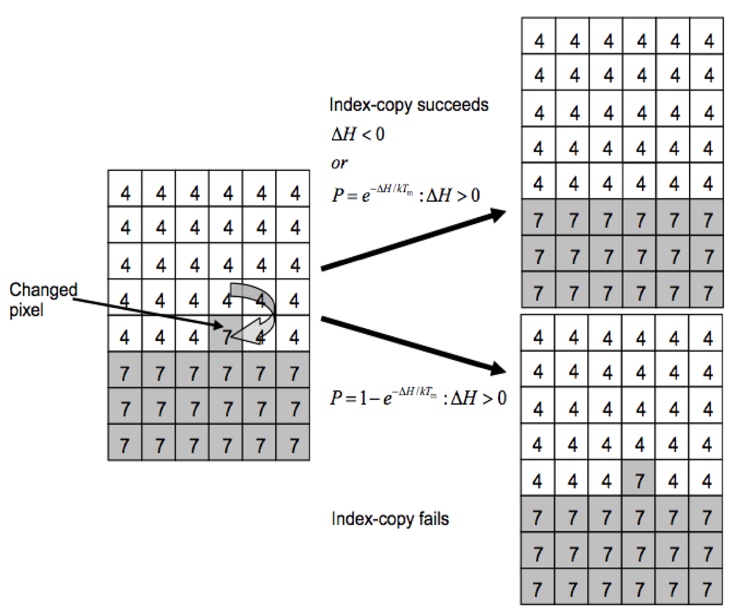

Fig. 3 : General model mechanism. A pixel belonging to the cell number 4 randomly try to replace of a pixel of cell number 7. This change will be accepted with a probability depending on the resulting system energy variation. Extracted from Swat *et al.*, Methods Mol Biol, 2009

Experimental data

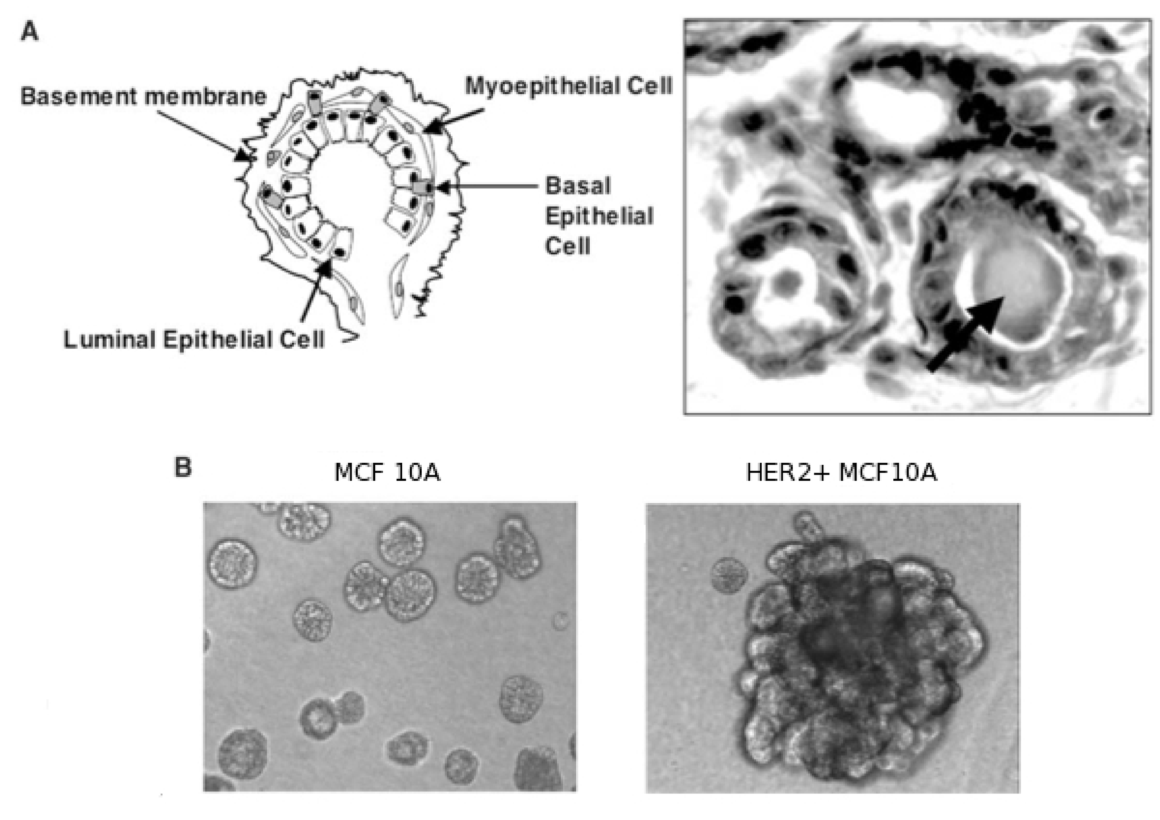

Fig. 1 : (A) view of mammary glands *in vivo*. (B) *In vitro* growth of wild and mutated MCF10A spheroids. Adapted from Debnath *et al.*, Methods, 2003

In vivo, mammary epithelial cells form monolayer spheroidic ducts (fig. 1.A). These structures are reproduced in vitro by growing MCF10A cells in 3D Matrigel medium. If the cells are transformed to over-express HER2 they form protruding, filled clusters comparable to ductal carcinoma in situ (fig. 1.B).
Volume, lumen size (Core Factor) and compactness (CTH and Solidity) of the structures were assessed using an image analysis software on cultures of spheroids of wild and mutated cells (fig. 2) [1].

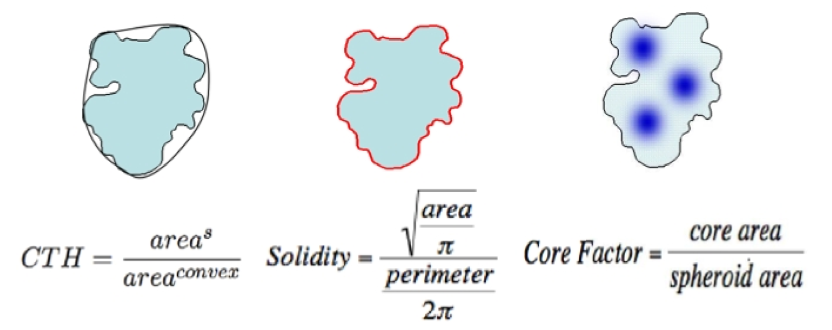

Fig. 2 : Shape measurements made on the spheroids grown *in vitro*. Extracted from Emde *et al*, Oncogene, 2011

Simulations

Depending on the length of their contacts with the others cells, the extracellular matrix and the lumen surrounding them, defined by 8 parameters, our virtual cells may change state and have their adhesion properties vary. The entrance in proliferation, polarization and apoptosis are conditioned by 3 probabilities.

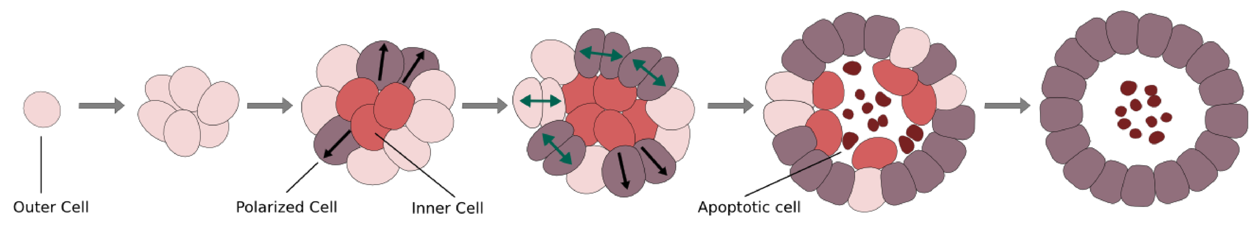

Fig. 4 : Schematic view of the virtual cells growth. Starting from an outer cell, proliferation leads to the change of type of cells depending on their positions in the cluster. Inner cells loosing contact with the ECM enter apoptosis while outer cells having sufficient contacts with theirs neighbours can polarize and stabilize.

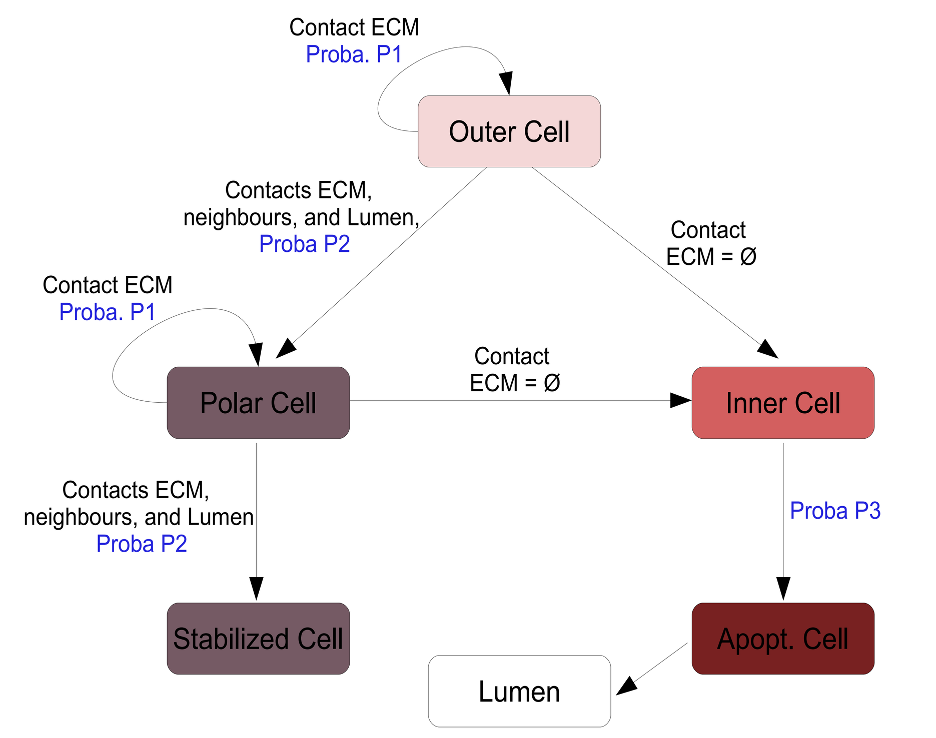

Fig. 5 : Simulation of the spheroid growth, graph of the type changing rules.

Results

1. Parameters identification for the wild phenotype

To find the parameters reproducing the wild phenotype, we compared measurements of conformation on simulations and on experimental data. A sensitivity analysis permitted to retrieve the most important parameters in respect to each aspect of the shape.

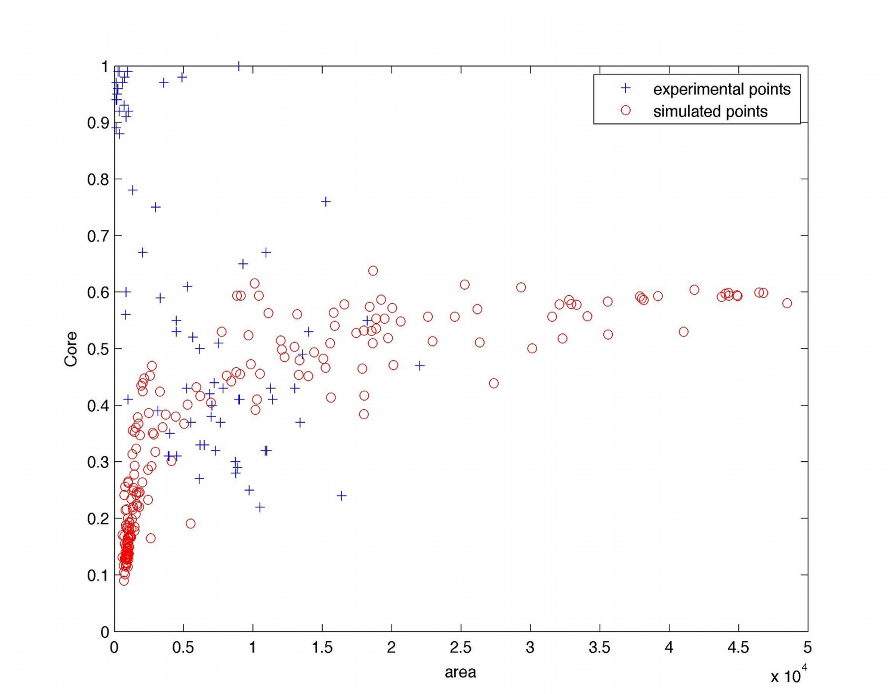

Fig. 6 : Comparison of the shape measurements on in vitro experiments and spheroids simulated from 200 random parameter sets.

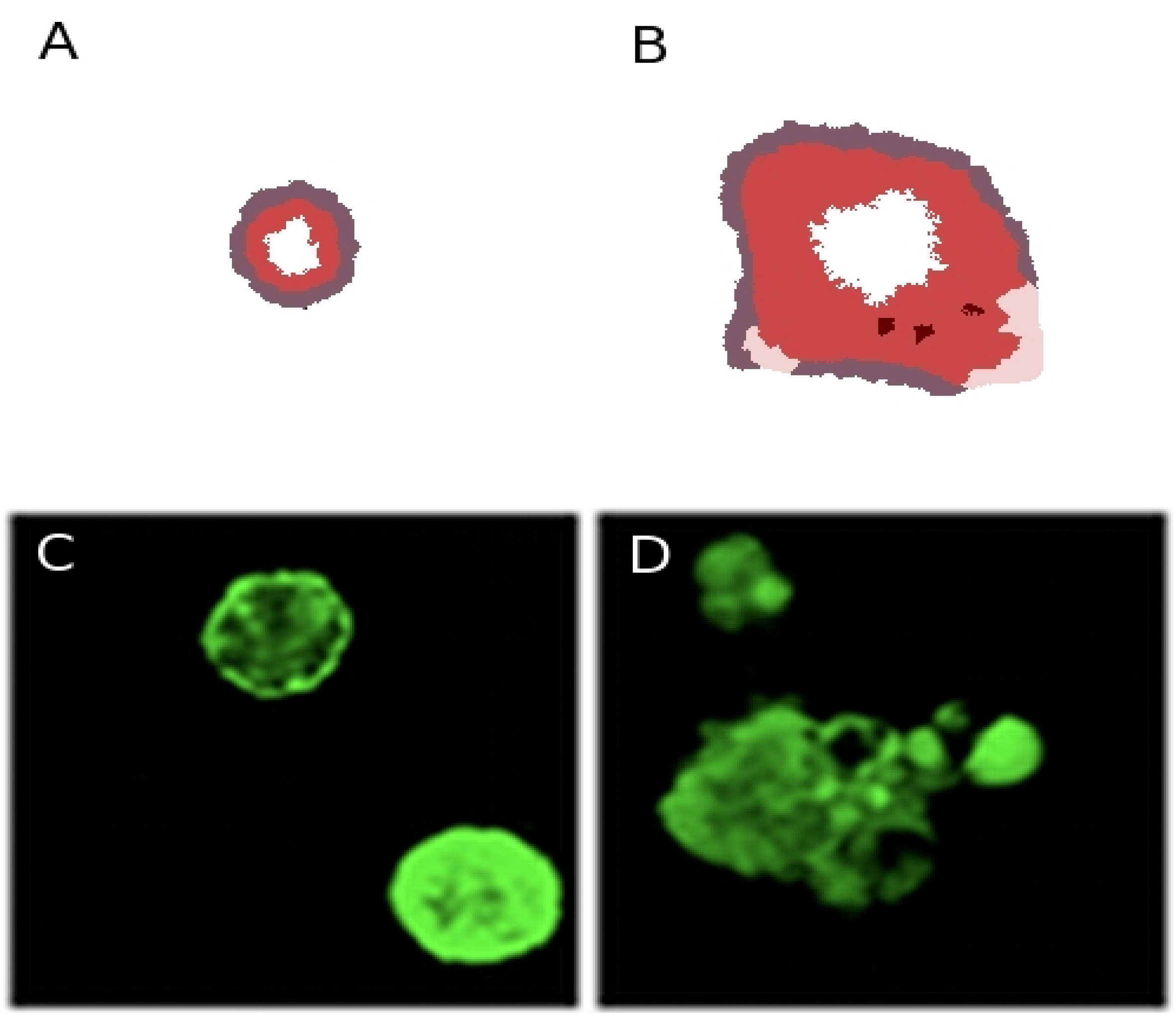

Fig. 7 : Qualitative comparison between simulations and experimental images. Spheroids simulated with the parameter sets leading to (A) a wild phenotype and (B) a mutated phenotype. Confocal image of in vitro grown spheroids of (C) normal cells, and (D) HER2 over-expressing cells

2. Derivation of the mutant phenotype

The mutated structures were retrieved by varying the probabilities of proliferation, polarization and apoptosis once the conditions triggering these changes were reached. The sensitivity analysis on these parameters concludes that a modification of all three properties is needed to reproduce the mutated phenotype. The probability of proliferation should be increased, while the probabilities of apoptosis and polarization should be decreased.

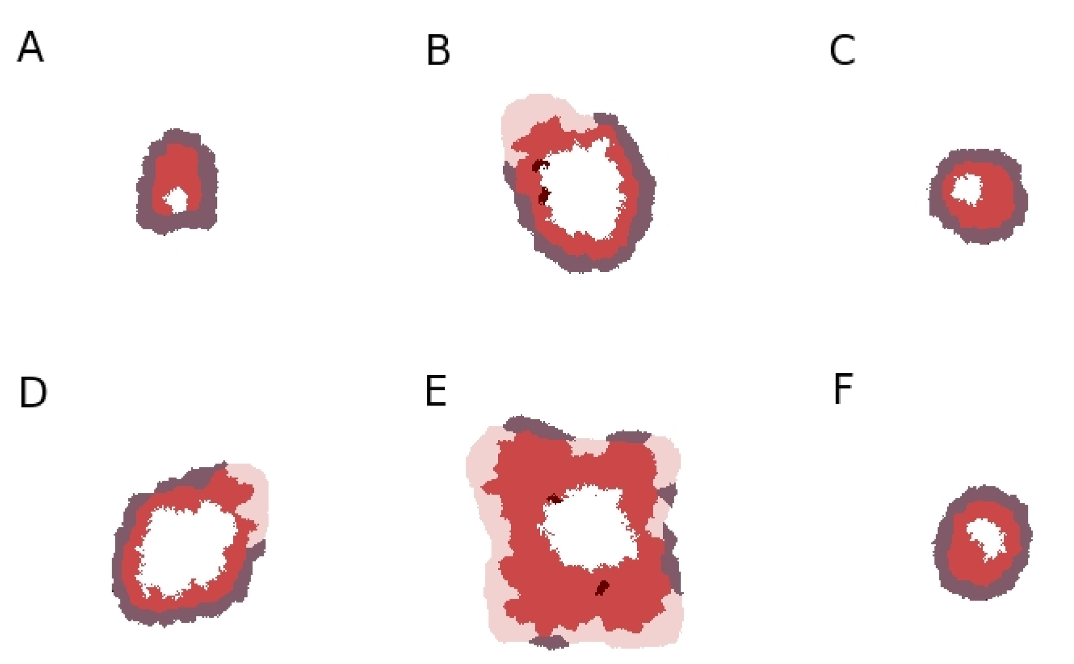

Fig. 9 : Simulations of spheroid growth made with the variation of the probabilities one by one : (A) proliferation, (B) polarization, (C) apoptosis ; or with the variation of couples of probabilities : (D) proliferation and polarization, (E) polarization and apoptosis, (F) proliferation and apoptosis.
Only the couple polarization+apoptosis permits to retrieve a phenotype close to the mutated phenotype observed in vitro. Nonetheless, the similarity is higher when all three probabilities are changed together.

Conclusions

We were able to reproduce the growth of spheroids of normal and HER2 over-expressing mammary epithelial cells. In the first case, the conformation depends on the contacts between the cells. And from this step, a simple modification of the probabilities of cellular processes to occur permits to retrieve the mutated phenotype. It is interesting to note that all three processes : proliferation, polarization and apoptosis must be taken into account. We can then infer that the HER2+ mutation has an effect on these three mechanisms. The next step of this project is to integrate the molecular pathways of HER2 signalling and simulate their blockade by targeted treatments. Comparison between such simulations and experimental data may give us an insight on links between molecular signals and cellular processes. Moreover it may give clues on optimal combinations of drugs.

References

[1] Emde *et al.* Combining epitope-distinct antibodies to HER2 : cooperative inhibitory effects on invasive growth, *Oncogene* 2011
[2] Izaguirre *et al.*- CompuCell, a multi-model framework for simulation and morphogenesis, *Bioinformatics* 2004
[3] Glazier and Graner, Simulation of the differential adhesion driven rearrangement of biological cells, *Physical Review E* 1993

For a more detailed information on CompuCell3D : www.compucell3d.org

ENS DE LYON — CWI — Netherlands Consortium for Systems Biology — Inria INVENTORS FOR THE DIGITAL WORLD